UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

007141

APR 25 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Dicamba: Oncogenicity Study in Mice with Dicamba Technical and a 21-Day Dermal Toxicity Study in Rabbits with Banvel Herbicide

TO: Taylor/Walters PM-25
Registration Division (H7505C)

FROM: K. Clark Swentzel 4/14/89
Acting Section Head
Toxicology Branch II (HFAS)
HED (H7509C)

THRU: Marcia van Gemert, Ph.D. 4/24/89
Acting Branch Chief
Toxicology Branch II (HFAS)
HED (H7509C)

EPA ID No.: 3F2794
Project No.: 9-0500
Caswell No.: 295
Registrant: Sandoz Corp.

ACTION REQUESTED: Review subject studies

CONCLUSIONS:

Oncogenicity Study:

Dicamba technical was administered to Charles-River CD-1 mice at dietary levels of 50, 150, 1000 and 3000 ppm for 89 weeks in males and 104 weeks in females. There was no significant biological evidence that dicamba induced a oncogenic response under the conditions of this study. Although a higher dosage level of dicamba would probably been appropriate for this study, the Agency previously approved 3000 ppm as the high-dose in a mouse oncogenicity study.

An equivocal LEL for systemic toxicity, based on increased mortalities in males and decreased body weight gain in females, is 3000 ppm (approximately 360 mg/kg/day). The NOEL is 1000 ppm (approximately 115 mg/kg/day).

Core-classification: minimum

21-Day Dermal Toxicity Study:

Banvel Herbicide was applied undiluted to the shaved intact dorsal skin of New Zealand white rabbits (5/sex/group) at daily doses of 40, 200 and 1000 mg/kg for 3 weeks (5 days/week = 15 or 16 applications).

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There was no convincing evidence that systemic toxicity was induced at any dose level, however, the high dose level may approximate a limit dose, depending on the purity of the test material.

Dose-related dermal irritation responses were observed at the application sites. Desquamation was seen predominantly in the 1000 mg/kg group while moderate erythema, moderate edema and atonia were observed exclusively in the 1000 mg/kg group. A dose-related incidence of fissuring was noted in the 200 and 1000 mg/kg groups. The severity of acanthosis and the incidence of hyperkeratosis was increased at these sites among rabbits in the 200 and 1000 mg/kg groups.

Therefore, the NOEL for dermal irritation is 40 mg/kg/day and the LEL is 200 mg/kg/day. The NOEL for systemic toxicity is 1000 mg/kg/day, the highest dose administered.

The registrant must submit the purity of the test material before this evaluation can be considered complete.

Core-classification: supplementary (can be upgraded if the purity of the Banvel Herbicide is provided)

Reviewed by: K. Clark Swentzel K. Clark Swentzel 3/30/89 007141
Section II, Tox. Branch II (H7509C)
Secondary reviewer: Marcia van Gemert, Ph.D. Marcia van Gemert 7/2/89
Tox. Branch II, (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity/Mouse TOX. CHEM. NO.: 295

MRID NO.: 408724-01

TEST MATERIAL: 2-methoxy-3,6-dichlorobenzoic acid

SYNONYMS: Dicamba

STUDY NUMBER(S): VCL 72/871205

SPONSOR: Sandoz Corp.

TESTING FACILITY: Huntingdon Research Centre, Ltd.

TITLE OF REPORT: Dicamba: Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice

AUTHOR(S): S. Crome, V. Stuart, A. Anderson, D. Crook, W. Gibson, E. Fish, D. Lewis & C. Gopinath

REPORT ISSUED: October 11, 1988

CONCLUSION: Dicamba technical was administered to Charles-River CD-1 mice at dietary levels of 50, 150, 1000 and 3000 ppm for 89 weeks in males and 104 weeks in females. There was no significant biological evidence of oncogenicity from ingestion of dicamba. A statistically significant increase in the mortality rate in high-dose (3000 ppm) males could not clearly be associated with treatment because a statistically significant increase was also observed in low-mid-dose (150 ppm) males. Also, decreased body weight gain and an increased ratio of lymphocytes to neutrophils in high-dose females could not be related to treatment with any degree certainty. Although the mice in this study might have tolerated a higher dosage level, based on the lack of systemic effects, 3000 ppm was previously approved by the Agency for a mouse oncogenicity study with dicamba, proposed by another registrant.

An equivocal LEL for systemic toxicity, based on increased mortalities in males and decreased body weight gain in females, is 3000 ppm (approximately 360 mg/kg/day). The NOEL is 1000 ppm (approximately 115 mg/kg/day).

Core classification: minimum

Quality Assurance Statement: included (signed and dated by QAU)

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A. MATERIALS:

1. Test compound: Dicamba technical, described as a light tan granular solid, batch # 52625110 , purity: 86.8%.

2. Test animals: Species: mouse, Strain: Charles River CD-1, Age: approximately 50 days old at study initiation, Weight: mean weights were approximately 25 and 20 g for males and females, respectively, Acclimation period: 23 days.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dietary level(ppm)	Mouse Numbers male	female
1 Cont.	0	52	52
2 Low (LD)	50	52	52
3 Low Mid (LMD)	150	52	52
4 High Mid (HMD)	1000	52	52
5 High(HDT)	3000	52	52
6 Health check(pre-test)	0	10	10

2. Diet preparation

The report indicated that the test material was administered by admixture with the diet without correction for the purity of the technical material, however data from the analyses of the diet mixtures (see below) do not corroborate this since compound levels typically exceeded 86.8% (purity) of nominal concentration. Control rats received normal untreated diet.

The required test material concentrations were prepared by direct dilution of the pre-mix, which was prepared weekly, with untreated diet and mixing in a double cone blender for a minimum period of 7 minutes. Fresh diet mixtures were prepared weekly and remained in the cage hopper for a maximum of 4 days.

Dicamba concentrations in the diet were determined in mixtures prepared for weeks 1, 13, 26, 39, 41, 52, 65, 78, 91 and 104 of the study as well as the pre-mix prepared for week 14. Diet mixtures containing low (50 ppm) and high (3000 ppm) concentrations of Dicamba were analysed for stability and homogeneity.

Results -

Dicamba concentrations were typically lower than the indicated nominal levels, however, the decrement exceeded 10% in only 7/72 measurements; 2 values were more than 10% higher than respective nominal levels.

Homogeneity examinations showed that test material levels were 98.8 to 113.0% of nominal for 50 ppm and 83.7 to 91.2% of nominal for 3000 ppm; the lowest level was measured in the bottom (location) sample in each case.

The stability test for diet preparations stored at room temperature and 4°C generated the following data:

Stability of Diet Preparations

Nominal concentration of Dicamba (ppm)	Analytical findings (% of nominal)	
	Day 0	Day 7
Ambient temp.		
50	97.2	102.6
3000	93.3	82.3
4°C		
50	97.2	105.6
3000	93.3	88.3

The investigator attributed the low values for the 3000 ppm samples in the homogeneity and stability tests to recovery problems encountered during the extraction procedure. The values obtained at day 0 in the stability test support the investigator's opinion.

3. Animal Maintenance

Animals received food (Labsure Laboratory Animal Diet No. 2) and water ad libitum.

The mice were housed by sex in groups of 4 in polypropylene cages, with autoclaved sifted sawdust as bedding. Each mouse was identified by cage number (tattooed on tail) and an ear mark.

The environmental parameters were: room temperature- 20-23°C; humidity- 44-58%; light cycle- 12 hours.

Ten mice/sex were randomly selected before initiation of the study as spare animals and were used for "health check" purposes. The inspection included hematology, gross necropsy and microscopic examinations of any abnormalities. Lungs, liver, kidneys, spleen and heart were preserved in fixative.

Results-

The investigator indicated that a high total WBC count with a low hemoglobin level was seen in one male, however, no clinical signs or gross abnormalities were noted for this animal. Enlarged spleens were noted in two males; microscopic examination of these organs revealed minimal extramedullary hematopoiesis. There was no evidence of infectious disease.

4. Statistics - Analysis of variance was used to assess the significance of intergroup differences in the food consumption and body weight data. Intergroup comparisons were carried out using Williams' test. The remaining statistical procedures used in this study are shown on appended page 1.

5. Quality assurance: a statement, which was signed and dated, was included in the report.

C. METHODS AND RESULTS:

1. Observations (mortalities and clinical signs)

The animals were examined twice daily in order to find dead and moribund mice. Moribund animals were killed by CO_2 asphyxiation and subjected to gross examination. The spectrum of tissues examined at termination was routinely preserved in buffered 10% formalin. Examinations for clinical signs were performed daily for the first 4 weeks, once weekly between weeks 4 and 40 and twice weekly from week 40 on. These examinations included palpation.

Results-

The mortality incidence data are shown in the following table:

Mortality Distribution

Group (ppm)	Mortalities (No./52)	Survival at Termination (%)
Males		
0	20@	62
50	28	46
150	34*	35
1000	21	60
3000	36*	31
Females		
0	22	58
50	24#	53
150	34*	35
1000	28	46
3000	26	50

* $p<0.05$, pairwise comparison; @ $p<0.05$, trend analysis: data for all groups
# No./51, one animal incorrectly sexed

These data show a statistically significant trend in increasing mortality among treated males (not significant if high-dose group is excluded) and a significant increase in mortalities among high-dose males, however, a significant increase was also observed in the low-mid-dose (150 ppm) males. The investigator summarized the "factors contributory to death" and the incidence of amyloidosis was higher than any other single factor among males that died in all groups, especially high-dose males. Amyloidosis was observed in liver, spleen, kidneys, heart as well as in other organs and tissues of these animals.

Incidence of Amyloidosis in Males that Died

	No. observed/No. that died (%)				
Group (ppm)--	0	50	150	1000	3000
	7/20(35)	7/28(25)	10/34(29)	5/21(24)	17/36(47)

The investigator did not note any clinical signs that could be readily associated with treatment.

2. Body weight

Body weights were recorded at the initiation of the study and once/week thereafter.

Results-

Inter-group comparisons of mean body weight gain are shown in the following table:

Body Weight Gain During Treatment

Group (ppm)	Body weight gain (g)	Percent of control
Males		
0	13.9	---
50	15.0	108
150	14.9	107
1000	14.3	103
3000	15.4	111
Females		
0	15.3	--
50	14.3	93
150	14.1	92
1000	14.5	95
3000	12.7	83

The only group which appeared to be affected by treatment was the high-dose females which had a 17% decrement in body weight gain compared to controls ($p=0.07$), however, the corresponding difference in absolute body weight was -7.7% (39g for controls vs. 36g for high-dose).

3. Food consumption and compound intake

Food consumption was calculated for each cage on a weekly basis. This number was divided by the number of mice alive in the cage to estimate individual consumption (g/mouse/week). These data plus dietary levels of dicamba were used to estimate test material consumption (mg/kg/day). Food utilization efficiency was calculated as food consumed per unit gain in body weight.

Results-

Food consumption was comparable between treated and control groups. The overall consumption values for treated males ranged from 95 to 106% of the control value and 97 to 106% of controls for treated females. Likewise, food utilization values for treated males and females were comparable to or exceeded those of respective controls (food consumption and utilization data shown on appended page 2).

The investigator calculated the following compound consumption values:

Mean compound consumption (mg/kg/day)

Group (ppm)	Males	Females
50	5.5	5.8
150	17.2	18.8
1000	108.0	121.0
3000	358.0	364.0

4. Hematology

Venous blood was collected from the tail vein and smears prepared from all mice killed during the study, if possible, and from all surviving mice in week 53, 80 and immediately prior to termination. These slides were fixed and stained. At weeks 53 and 80 and at termination, a differential white blood cell count and a subjective assessment of the presence or absence of leukemia was performed on smears from 10 males and 10 females from the high-dose and control groups. At termination, as differences were observed between control and treated females, this examination was extended to all surviving females in all groups.

Results-

The investigator found that differential white blood cell counts on blood smears prepared prior to termination from 10 control females and 10 high-dose females revealed a marked decrease in the proportion of neutrophils and an increase in the proportion of lymphocytes among high-dose

mice. This was further investigated by examining blood smears from all of the other female groups and a similar change was observed in the 1000 and 150 ppm groups (appended page 3). However, this change in cell proportions was not dose-related and the neutrophil/lymphocyte proportions were comparable to those observed in control females at 53 and 80 weeks (appended pages 4 & 5). Therefore, the noted changes do not appear to be toxicologically significant.

5. Sacrifice and Pathology -

Male mice were killed following 89 completed weeks of treatment when the survival approached 30% in the males receiving 150 or 3000 ppm. Female mice were killed following 104 weeks of treatment when survival was at least 35% in all groups.

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The organs CHECKED (XX) were weighed.

		Digestive system			Cardiovasc./Hemat.			Neurologic
		Tongue		X	.Aorta*	X	X	.Brain*†
		.Salivary glands*		X	.Heart*		X	Periph. nerve*#
	X	.Esophagus*			.Bone marrow*		X	Spinal cord (3 levels)*#
	X	.Stomach*		X	.Lymph nodes*		X	.Pituitary*
	X	.Duodenum*		X	.Spleen*		X	Eyes (optic n.)*#
	X	.Jejunum*		X	.Thymus*			Glandular
	X	.Ileum*			Urogenital		X	.Adrenals*
	X	.Cecum*		X	X	.Kidneys*†		Lacrimal gland#
	X	.Colon*			X	.Urinary bladder*	X	Mammary gland*#
	X	.Rectum*		X	X	.Testes*†	X	.Parathyroids*††
X	X	.Liver*†			X	Epididymides	X	.Thyroids*††
	X	Gall bladder*#			X	Prostate		Other
	X	.Pancreas*			X	Seminal vesicle	X	Bone*#
		Respiratory			X	Ovaries*†	X	Skeletal muscle*#
	X	.Trachea*			X	.Uterus*	X	Skin*#
	X	.Lung*					X	All gross lesions and masses*
		Nose°						
		Pharynx°						
		Larynx°						

* Required for subchronic and chronic studies
° Required for chronic inhalation
In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

The microscopic examinations consisted of the following:

1) All designated tissues from all mice in the control and high-dose groups dying during the study or killed at termination.

2) All indicated tissues from all mice of the intermediate and low dosage groups dying during the study.

3) Any macroscopically abnormal tissue from mice of the intermediate and low dosage level groups dying during the study.

4) Lung, liver and kidney from all mice in the low and intermediate dosage level groups dying during the study or killed at termination.

Results-

a. Organ weight

There was a significant increase ($p<0.05$; Williams' test) in mean kidney weight in the treated female groups receiving 1000 and 3000 ppm of test material, however, there were no related histomorphological changes noted. Kidney weights in males had no apparent relationship to treatment. Also, none of the other intergroup differences in organ weights appeared to be associated with treatment.

b. Gross pathology

Gross examination of mice that died or were killed in extremis or at termination did not reveal any changes that were clearly associated with treatment. Although the incidence of enlarged spleen increased in both treated males and females (37% in high-dose groups for both sexes) compared to respective controls (21% for both sexes), none of the observed increases were statistically significant by pairwise comparison and the increased values were not dose-related.

c. Microscopic pathology

1) Non-neoplastic

The observed fluctuations in the investigated parameters (eg., increased incidences of extramedullary hematopoiesis in the spleen of treated males and granulomatous inflammation in the liver of treated females: appended pages 6 & 7) were either not dose-related or not statistically significant, therefore, association with treatment was not apparent in the data generated for these parameters.

2) Neoplastic

There was an increased incidence of lymphoid tumors (lymphosarcoma excluding pleomorphic lymphosarcoma) in treated females (appended page 8). The combined incidences of this tumor in mice that died and those that were killed at termination were:

Lymphosarcoma in Female Mice: no. with tumors/no. examined (%)

Group (ppm)	0	50	150	1000	3000
	2/52 (3.8)	4/51 (7.7)	8/52* (15.4)	7/52 (13.5)	5/52 (9.6)

* $p < 0.05$, Fisher's Exact

Spontaneous incidence in this laboratory (mean incidence in controls from 9 studies): 6 - 33%.

The increased incidence of these tumors in treated females is not clearly associated with treatment since the group incidences are not dose-related and they are within the noted historical range. Additionally, the incidence in the concurrent control is below the historical range.

Examination of the remaining histologic data generated in this study did not reveal a neoplastic response in any of the tissues/organs examined in treated mice.

D. DISCUSSION:

Although there was no significant biological evidence of an oncogenic response from dicamba in this study, the animals should possibly have been tested at a higher dose level.

The mortality incidence was significantly high ($p < 0.05$) in high-dose (3000 ppm) males (with a statistically significant trend analysis), however, the statistically significant increase in mortality in 150 ppm males makes the results observed in high-dose males equivocal with regards to a treatment-related effect. The only "factor contributory to death", indicated by the investigator, which could clearly be associated with high-dose males that died, was amyloidosis.

Although body weight gain was decreased in 3000 ppm females, the difference from terminal mean control weights was less than 10%.

An increased ratio of lymphocytes to neutrophils in 3000 ppm females could not be conclusively associated with treatment.

Macroscopic examinations performed during necropsy and histologic examination of noted tissues did not reveal evidence of systemic toxicity.

Although evidence that an MTD was attained in this study is equivocal, the Agency previously approved 3000 ppm as a high dose for a mouse oncogenicity study proposed by another registrant (EPA Memorandum, April, Toxicology Branch, to Taylor/Walters, Registration Division, November 15, 1984).

E. SUMMARY:

Dicamba technical was administered to Charles-River CD-1 mice at dietary levels of 50, 150, 1000, and 3000 ppm for 89 weeks in males and 104 weeks in females. There was no significant biological evidence of oncogenicity from ingestion of dicamba. A statistically significant increase in the mortality rate in high-dose (3000 ppm) males could not clearly be associated with treatment because a statistically significant increase was also observed in low-mid-dose (150 ppm) males. Also, decreased body weight gain and an increased ratio of lymphocytes to neutrophils in high-dose females could not be related to treatment with any degree certainty. Although the mice in this study might have tolerated a higher dosage level, based on the lack of systemic effects, 3000 ppm was previously approved by the Agency for a mouse oncogenicity study with dicamba, proposed by another registrant.

F. CONCLUSION:

Dietary levels of dicamba up to 3000 ppm (approximately 360 mg/kg/day) did not induce oncogenicity in male and female Charles River CD-1 mice under the conditions of this test. An equivocal LEL for systemic toxicity, based on increased mortalities in males and decreased body weight gain in females, is 3000 ppm. The NOEL is 1000 ppm (approximately 115 mg/kg/day).

Core classification: minimum

Statistical analysis of food intake data was performed using total food intake for each cage of animals (g/animal) over specified time periods. These totals were calculated using the exact weekly figures for each cage. Bodyweight data were analysed using the weight gains of those mice surviving throughout specified time periods.

Attention was also given to tumour size, tumour multiplicity and tumour growth rate by the pathologist.

Mortality was analysed using logrank methods (1, 2).

The following sequence of statistical tests was used for food consumption, bodyweight, blood smear and organ weight data:

(i) Where the data consisted predominantly of one particular value (relative frequency of the mode exceeds 75%), the proportion of animals with values different from the mode was analysed using Fisher's Exact test (3) or Mantel's test (4). Fisher's test was used to detect general differences between treatments while Mantel's test was used specifically to detect dose-related trends in the numbers of such animals. Otherwise:

(ii) Bartlett's test (5) was applied to test for heterogeneity of variance between treatments. Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.

(iii) If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks (6) was used.

(iv) Analyses of variance were followed by Student's 't' test and Williams' test (8) for a dose-related response although only the one thought most appropriate for the response pattern observed was reported. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of the 't' test and Williams' test (Shirley's test, (7)).

Organ weights were subjected to analysis of variance or analysis of covariance (9). Analysis of covariance was carried out using final bodyweight as covariate when the within-group relationship between organ weight and bodyweight was significant at the 10% level. A log (x+1) transformation of organ weight (or of both bodyweight and organ weight) was used if significant (1% level) heterogeneity of variance was revealed in the organ weight (bodyweight) data by Bartlett's test (2), and if the transformed data showed less heterogeneity of variance than the untransformed data.

Data on the numbers of animals with tumours were analysed by the time-to-tumour methods recommended by the IARC (10).

FOOD CONSUMPTION (Table 3, Appendix 2)

Total food consumption during treatment was as follows:

Group/dosage ppm		Total food consumption during Weeks:							
		1-52	% of control	53-78	% of control	79-T	% of control	1-T	% of control
1♂	Control	1735	-	845	-	373	-	2940	-
2♂	50	1700	98	891	105	384	103	2945	100
3♂	150	1767	102	869	103	390	105	3000	102
4♂	1000	1641	95	791	94	360	97	2792	95
5♂	3000	1787	103	911	108	422*	113	3109	106
1♀	Control	1456	-	755	-	775	-	2985	-
2♀	50	1431	98	717	95	743	96	2886	97
3♀	150	1492	102	782	104	866	112	3153	106
4♀	1000	1468	101	766	101	793	102	2992	100
5♀	3000	1447	99	731	97	760	98	2938	98

T Last completed week prior to terminal kill
Level of significance: * 0.05≥P>0.01 in comparison with controls (Williams' test)

Overall food intake by treated male and female mice was similar to that of controls. Differences which occurred were not associated with any dose-related trends and were considered to be unrelated to treatment with Dicamba.

EFFICIENCY OF FOOD UTILISATION (Table 4)

The efficiency of food utilisation as expressed by the food conversion ratio (FCR) was similar for control and treated mice over the first 26 weeks of treatment, the period of fastest growth.

Group/dosage ppm		Food conversion ratio
		Weeks 1-26
1♂	Control	79.0
2♂	50	80.0
3♂	150	75.8
4♂	1000	69.2
5♂	3000	84.1
1♀	Control	85.1
2♀	50	88.3
3♀	150	89.1
4♀	1000	85.9
5♀	3000	89.0

TABLE 6

(Haematology - continued)

Week 105 (24 July 1987)

Group/ dosage ppm	Number of animals	WBC %				
		N	L	E	B	M
1♀ Control	31	46	52	2	0	0
2♀ 50	27	45	53	1	0	0
3♀ 150	18	** 33	** 66	* 1	0	0
4♀ 1000	24	** 31	** 69	* 1	0	0
5♀ 3000	26	** 34	** 65	* 1	0	0

Level of significance: * 0.05≥P>0.01) in comparison
(Williams' test) ** 0.01≥P) with controls

TABLE 6

Haematology - group mean values

Week 53 (19-22 May 1986)

Group/ dosage ppm	WBC %				
	N	L	E	B	M
1♂ Control	53	46	1	0	0
5♂ 3000	44	55	1	0	0
1♀ Control	34	64	3	0	0
5♀ 3000	35	64	1	0	0

Level of significance (Student's 't' test) : No significant differences from controls noted (P>0.05)

Female eosinophil data were analysed using a distribution-free Williams' test

Female monocyte data were analysed using frequency analysis methods

10 mice per group per sex examined

TABLE 6

(Haematology - continued)

Week 80 (24-27 November 1986)

Group/ dosage ppm	WBC X				
	N	L	E	B	M
1♂ Control	30	68	2	0	0
5♂ 3000	31	66	2	0	0
1♀ Control	30	68	2	0	0
5♀ 3000	38	61	2	0	0

Level of significance : No significant differences from controls noted (P>0.05)
(Student's 't' test)

Male monocyte data analysed using frequency analysis methods
10 mice per group per sex examined

TABLE 12 VCL/72

(Non-neoplastic morphology incidence summary of male mice dying or killed during the study and killed at termination - continued)

Number of Male Animals with:	1		2		3		4		5	
	D	T	D	T	D	T	D	T	D	T
Animals Logged:	20	32	28	24	34	18	21	31	36	16
Biliary cysts	0	1	0	0	0	0	0	0	0	0
Minimal autolysis	1	0	1	0	5	0	4	0	1	0
Moderate autolysis	1	0	4	0	2	0	0	0	3	0
Liver ORO	20	32	28	0	34	0	21	0	36	16
Not Remarkable	15	12	25	0	31	0	17	0	28	8
Missing	0	0	0	0	0	0	0	0	1	0
Fat in periportal hepatocytes	0	0	1	0	1	0	1	0	2	1
Fat in centrilobular hepatocytes	1	9	1	0	1	0	3	0	4	2
Fat in hepatocytes	4	11	1	0	1	0	0	0	1	4
Altered hepatocytes	0	0	0	0	0	0	0	0	0	1
Gall bladder	20	32	28	0	34	0	21	0	36	16
Not Remarkable	14	30	14	0	22	0	12	0	23	16
Missing	1	0	1	0	1	0	0	0	2	0
Autolysis	4	0	9	0	7	0	9	0	7	0
Distended	0	0	1	0	0	0	0	0	0	0
Dilated	0	2	0	0	1	0	0	0	0	0
Amyloidosis	0	0	0	0	1	0	0	0	0	0
Vacuolation of epithelium	0	0	0	0	0	0	0	0	1	0
Minimal autolysis	0	0	3	0	0	0	0	0	3	0
Moderate autolysis	0	0	0	0	1	0	0	0	0	0
Spleen	20	32	28	3	34	4	21	2	36	16
Not Remarkable	6	22	1	0	7	0	3	0	4	10
Autolysis	1	0	1	0	0	0	1	0	0	0
Amyloidosis	3	1	6	0	9	1	5	0	10	1
Increased cellularity of white pulp	3	8	2	0	4	3	4	1	6	4
Decreased cellularity of white pulp	0	0	6	0	0	0	0	0	2	0
Lymphocytolysis in white pulp	1	0	1	0	0	0	1	0	0	0
Decreased cellularity of red pulp	0	0	0	0	1	0	0	0	0	0
Histiocytosis	1	0	0	0	0	0	0	0	0	0
Extramedullary haemopoiesis	6	3	11	1	12	4	9	1	18	1
Minimal autolysis	1	0	2	0	2	0	1	0	1	0
Moderate autolysis	0	0	2	0	3	0	0	0	4	0
Pancreas	20	32	28	1	34	0	21	1	36	16
Not Remarkable	11	27	17	0	23	0	16	0	25	16
Missing	0	0	0	0	0	0	0	0	1	0
Autolysis	1	0	2	0	2	0	1	0	1	0
Pancreatitis	0	0	0	0	1	0	1	0	1	0
Perivascular lymphoid aggregates	1	2	0	0	0	0	0	0	0	0
Acinar hyperplasia	1	1	0	0	0	0	0	0	0	0

D : Animals killed or dying during study T : Terminals

TABLE 12 VCL/72

(Non-neoplastic morphology incidence summary of female mice dying or killed during the study and killed at termination - continued)

Number of Female Animals With:	1		2		3		4		5	
	D	T	D	T	D	T	D	T	D	T
Animals Logged:	22	30	24	27	34	18	28	24	26	26
Liver	22	30	24	27	34	18	28	24	26	26
Not Remarkable	4	1	5	4	2	2	0	2	2	2
Autolysis	1	0	1	0	1	0	0	0	1	0
Basophilic hepatocytes	0	2	0	0	1	0	0	3	0	3
Eosinophilic hepatocytes	0	0	0	0	1	1	0	0	0	3
Rarefied hepatocytes	0	0	1	0	1	0	0	0	0	0
Vacuolated hepatocytes	0	1	1	0	0	0	0	0	0	0
Hepatocyte necrosis	2	2	0	1	3	0	5	0	4	1
Hepatocyte degeneration	1	4	0	4	2	0	0	6	2	2
Granulomatous inflammation	0	1	2	13	2	0	0	7	3	13
Inflammatory cells in sinusoids	0	0	0	0	0	0	0	0	1	0
Mononuclear cell foci	3	15	3	5	6	3	5	4	5	4
Centrilobular hepatocyte enlargement	0	0	0	1	0	0	0	1	0	0
Portal amyloidosis	5	3	4	1	10	1	12	1	5	2
Amyloidosis	0	0	0	1	0	0	0	0	0	0
Extramedullary haemopoiesis	6	2	5	0	4	0	6	0	8	0
Centrilobular hepatocyte vacuolation	1	20	2	7	2	7	4	10	0	10
Periportal hepatocyte vacuolation	0	0	0	0	0	0	1	0	0	0
Hepatocyte vacuolation	1	0	0	4	3	0	4	0	0	0
Angiectasis	0	2	0	0	0	0	0	1	1	0
Dilated congested sinusoids	1	0	0	0	1	0	0	0	0	0
Apparent capsular necrosis and inflammation	0	0	1	0	0	0	0	0	0	0
Fibrosis	0	0	0	0	0	0	0	0	1	0
Fibrous scarring	0	0	0	1	0	0	0	0	0	0
Capsular thickening	0	0	0	0	0	0	0	0	1	0
Cyst	0	0	1	0	0	0	0	0	0	0
Apparent increased cellularity	0	0	0	0	0	0	2	0	0	0
Thrombus(i)	0	1	0	0	0	0	0	0	0	0
Congestion	0	0	0	1	0	1	0	1	0	0
Biliary cysts	0	0	0	1	0	0	0	0	0	0
Pigmented Kupffer cells	0	3	0	2	0	1	2	1	2	7
Pigmented macrophages	0	0	0	0	0	1	0	0	0	0
Minimal autolysis	2	0	2	0	2	0	2	0	0	0
Moderate autolysis	0	0	1	0	6	0	1	0	3	0
Liver ORO	22	30	24	0	34	0	28	0	26	26
Not Remarkable	17	23	17	0	21	0	23	0	23	20
Autolysis	0	0	1	0	0	0	0	0	0	0
Fat in periportal hepatocytes	2	0	0	0	4	0	1	0	0	0
Fat in centrilobular hepatocytes	1	1	0	0	1	0	2	0	1	0
Fat in hepatocytes	2	6	6	0	8	0	2	0	2	6

D : Animals killed or dying during study T : Terminals

A table of data from control groups of studies recently completed in this laboratory is given below, together with data from VCL/72.

TABLE A

Lymphoid tumours in VCL/72 - females

Dose group	Control		50 ppm		150 ppm		1000 ppm		3000 ppm	
Number of female mice with:	D	T	D	T	D	T	D	T	D	T
Lymphosarcoma	1	1	3	1	7	1	4	3	3	2
Lymphoid leukaemia	0	0	0	0	1	0	0	0	0	0
Pleomorphic lymphosarcoma*	1	0	0	1	2	0	1	1	2	0
Number of mice examined	22	30	24	27	34	18	28	24	26	26

* The classification of lymphoid tumours has been changed in current studies. Pleomorphic lymphosarcoma was previously included in lymphosarcoma

TABLE B

Lymphoid tumours in untreated CD-1 mice - females

Study code	81E		82A		82B		82C		82D		82E	
Number of female mice with:	D	T	D	T	D	T	D	T	D	T	D	T
Lymphosarcoma	3	2	6	7	11	7	2	1	13	4	11	6
Lymphoid leukaemia	0	0	2	0	5	0	1	0	1	0	1	0
Number of mice examined	23	29	46	58	64	40	27	25	44	43	31	21

Study code	82F		83A		83B	
Number of female mice with:	D	T	D	T	D	T
Lymphosarcoma	6	7	6	1	5	1
Lymphoid leukaemia	1	0	3	0	1	0
Number of mice examined	29	23	24	28	32	20

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Reviewed by: K. Clark Swentzel K. Clark Swentzel 4/14/89
Section II, Tox. Branch II (H7509C)
Secondary reviewer: Marcia van Gemert, Ph.D. M. van Gemert 4/18/89
Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 21-Day Dermal Toxicity/Rabbit TOX. CHEM. NO.: 295

MRID NO.: 405479-01

TEST MATERIAL: 2-methoxy-3,6-dichlorobenzoic acid

SYNONYMS: Dicamba, Banvel

STUDY NUMBER(S): WIL-15163

SPONSOR: Sandoz Corp.

TESTING FACILITY: W.I.L. Research Laboratories, Inc.

TITLE OF REPORT: 21-Day Dermal Toxicity Study in Rabbits with Banvel Hervicide

AUTHORS: J.A. Strouse and Dennis J. Nass

REPORT ISSUED: February 26, 1986

CONCLUSION:

Banvel Herbicide was applied undiluted to the shaved intact dorsal skin of New Zealand white rabbits (5/sex/group) at daily doses of 40, 200 and 1000 mg/kg for 3 weeks (5 days/week = 15 or 16 applications).

There was no convincing evidence that systemic toxicity was induced at any dose level, however, the high dose level may approximate a limit dose, depending on the purity of the test material.

Dose-related dermal irritation responses were observed at the application sites. Desquamation was seen predominantly in the 1000 mg/kg group while moderate erythema, moderate edema and atonia were observed exclusively in the 1000 mg/kg group. A dose-related incidence of fissuring was noted in the 200 and 1000 mg/kg groups. The severity of acanthosis and the incidence of hyperkeratosis was increased at these sites among rabbits in the 200 and 1000 mg/kg groups.

Therefore, the NOEL for dermal irritation is 40 mg/kg/day and the LEL is 200 mg/kg/day. The NOEL for systemic toxicity is 1000 mg/kg/day, the highest dose administered.

The registrant must submit the purity of the test material before this evaluation can be considered complete.

Core-classification: supplementary (can be upgraded if the purity of the Banvel Herbicide is provided)

Quality Assurance Statement: a statement was signed and dated

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A. MATERIALS:

1. Test compound: Banvel Herbicide (SP-045-85), lot no. 52410301, described as a dark amber liquid with a specific gravity of 1.18 g/ml (purity not given), supplied by Velsicol Chemical Corporation. The report indicated that the test material is stable when stored in a sealed container at room temperature; no stability data were included.

2. Test animals: Species: rabbit; Strain: New Zealand white; Age: not given; Weight: 1958 to 2352 g at study initiation; Acclimation period: 15 days.

B. STUDY DESIGN:

1. Animal assignment

Animals (5/sex) were assigned randomly assigned to 1 control and 3 treatment groups. Animals were identified uniquely by plastic ear tags which displayed respective animals.

2. Animal maintenance

The test animals were housed individually in wire-mesh cages suspended above cage board. Room temperature was maintained at 67 ± 3°F with a minimum relative humidity of 40%. Ten fresh air changes were provided per hour; a 12 hour light cycle was provided. Food (Purina Certified Rabbit Chow #5322) and water were provided ad libitum except during periods of fasting prior to blood collection.

3. Administration of test material

The test material was applied undiluted to the shaved intact dorsal skin of each test animal for 5 days/week for 3 weeks for a total of 15 or 16 applications. The dosage levels were as follows:

Group	Material	Dosage level (mg/kg/day)	Dosage volume (ml/kg/day)
1	deionized water	0	0.85
2	Banvel	40	0.034
3	Banvel	200	0.17
4	Banvel	1000	0.85

The test material covered approximately 5, 10 and 25% of the total body surface for the 40, 200 and 1000 mg/kg/day groups, respectively. Individual doses were calculated based on the body weight recorded prior to test material application.

The test material was applied by inunction over the test site using a syringe and a glass rod. The test sites were then wrapped with a gauze binder, occluded with plastic wrap and secured with tape. All rabbits wore Elizabethan collars

to prevent ingestion of the test material and/or wrappings. At the end of the six-hour exposure period, the dressings were removed and the test sites were wiped with moistened disposable paper towels.

C. METHODS AND RESULTS:

1. Observations (mortalities and clinical signs)

All animals were examined weekly and on the day of sacrifice. They were observed for mortality and signs of overt toxicity twice daily for the duration of the study.

Results:

None of the animals died during the study. Clinical signs noted by the investigator (eg. urogenital matting as well as nasal and ocular discharge) did not appear to be related to treatment.

2. Dermal irritation

Application sites were examined for erythema, edema and other dermal findings once daily throughout the study period. Erythema and edema were evaluated based on a 4-step grading system of very slight, slight, moderate and severe.

Results:

Slight erythema and edema were observed sporadically in treated animals during the initial 4 days of the study, however, dose-related responses were noted from day 5 until the end of the study (Appended pages 1 through 4 from the report). Desquamation was seen predominantly in the 1000 mg/kg group while moderate erythema, moderate edema and atonia were observed exclusively in the 1000 mg/kg group. A dose-related incidence of fissuring was noted in the 200 and 1000 mg/kg groups.

3. Body weights:

Individual body weights were obtained on the day before study initiation, at weekly intervals and at termination.

Results:

Body weight gains were typically decreased in treated rabbits (Appended pages 5 and 6 from the report), however, the decreases were not dose-related, with the exception of the week 2 - 3 interval in females in which the decrease was statistically significant in high-dose females. The terminal body weight decrements between high-dose males and females and the respective concurrent control weights were only 8 and 4%, respectively (Appended pages 7 and 8 from the report). Considering these data, as well as the observed positive and negative individual body weight fluctuations, it can not be concluded with certainty that treatment had any effect on body weights.

4. Food consumption:

Food intake was based on a daily subjective estimation and was recorded as normal, decreased or increased. Therefore, any possible effect of treatment on food consumption can not be determined with any degree of confidence.

5. Clinical laboratory studies:

Clinical laboratory parameters were measured for 10 male and 10 female rabbits selected randomly prior to study initiation and on all animals prior to study termination. Blood was collected from the marginal ear vein following an overnight fasting period.

a. Hematology parameters:

Hematocrit
Hemoglobin
RBC count
WBC count
Platelet count
Mean corpuscular volume
Mean corpuscular hemoglobin concentration
Mean corpuscular hemoglobin
Differential WBC count - percent
- Unsegmented neutrophil
- Segmented neutrophil
- Lymphocyte
- Monocyte
- Eosinophil
- Basophil

Results:

There was no apparent association between observed hematologic changes and treatment.

b. Serum chemistry parameters:

Blood creatinine
Serum urea nitrogen
Albumin
Serum glutamic pyruvic transaminase (SGPT)
Serum glutamic oxaloacetic transaminase (SGOT)
Serum alkaline phosphatase
Total bilirubin
Globulin
Sodium
Calcium
Potassium
Total protein
Total cholesterol
Chloride
Albumin/Globulin Ratio
Phosphorus
Glucose

Results:

Observed changes were typically sporadic and unrelated to dose level of test material; none of the changes in measured parameters appeared to be associated with treatment.

6. Sacrifice and pathology:

a. Gross examination:

All animals sacrificed in extremis and at study termination were necropsied for macroscopic examinations. The necropsies included examination of the external surface, all orifices, the cranial cavity, the external and cut surfaces of the brain and spinal cord and the thoracic, abdominal and pelvic

cavities, including viscera. The following tissues and organs were preserved in 10% neutral buffered formalin:

Adrenals
Aorta
Bone with marrow(sternebrae)
Brain (3 areas)
Eyes with optic nerve
Gallbladder
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Cecum
  Colon
  Rectum
Heart
Kidneys
Liver(2 lobes)
Lungs(including bronchi)
Lymph node(mesenteric)
Ovaries with oviducts
Pancreas
Peripheral nerve(sciatic)
Pituitary
Prostate
Salivary gland(submaxillary)
Seminal vesicles
Skeletal muscle(vastus medialis)
Skin (treated and untreated)
Spinal cord(cervical)
Spleen
Testes with epididymides
Thymus
Thyroid gland(with parathyroids if present)
Trachea
Urinary bladder
Uterus with cervix and vagina
All gross lesions

Results:

The data in the report did not reveal gross changes which were related to treatment. "Thickened skin" was observed at the treated sites, however, this observation was noted in control as well as treatment groups.

b. Organ weights:

Kidneys, liver, ovaries and testes were removed from animals sacrificed at termination and weighed.

Results:

Slight but dose-related decreases in mean absolute and relative liver weights were observed in both treated males and females (Appended pages 9, 10, 11 and 12), however, none of the decreases was statistically significant (Dunnett's test). There was no histologic or biochemical evidence of treatment-related liver changes, therefore, the noted decreases in weight do not appear to be biologically significant. Also, the noted fluctuations in body weight during the study make the interpretation of these data more difficult.

No other organ weight changes were indicative of a treatment-related effect.

c. Microscopic examination:

Treated and untreated skin samples from all groups and liver and kidney samples from the low and high dose groups were examined microscopy.

Results:

The examination of application sites showed that a comparable incidence of acanthosis occured in all groups but it was somewhat more severe in the 200 and 1000 mg/kg groups (Appended page 13 from the report), whereas the predominant incidence of hyperkeratosis was seen in the 200 and 1000 mg/kg groups. Minimal to moderate portal mononuclear cell infiltrate was observed in the liver ot 3 high dose animals (1 male; 2 females); centrilobular hepatocytic vacuolation was seen in 1 female at this dose level.

One male and 1 female receiving 1000 mg/kg/day had mild chronic interstitial nephritis.

The noted observations in liver and kidneys were probably spontaneous in nature; the low incidences of these observations negate a clear association with treatment. The only observations which were obviously related to the administration of test material were the increased severity of acanthosis and the incidence of hyperkeratosis in animals receiving 200 and 1000 mg/kg/day.

7. Statistics:

All analyses were conducted using the two-tailed tests for a minimum significance level of 5% comparing the treatment groups to the venicle control group by sex. Analysis of body weights, body weight changes, clinical laboratory values and absolute and relative organ weights were analyzed ay a one-way analysis of variance, followed by Dunnett's Test.

D. SUMMARY:

Banvel Herbicide was applied undiluted to the shaved intact dorsal skin of New Zealand white rabbits (5/sex/group) at daily doses of 40, 200 and 1000 mg/kg for 3 weeks (5 days/week = 15 or 16 applications).

There was no convincing evidence that systemic toxicity was induced at any dose level, however, the high dose level may approximate a limit dose, depending on the purity of the test material.

Dose-related dermal irritation responses were observed at the application sites. Desquamation was seen predominantly in the 1000 mg/kg group while moderate erythema, moderate edema and atonia were observed exclusively in the 1000 mg/kg group. A dose-related incidence of fissuring was noted in the 200 and 1000 mg/kg groups. The severity of acanthosis and the incidence of hyperkeratosis was increased at these sites among rabbits in the 200 and 1000 mg/kg groups. Therefore, the NOEL for dermal irritation is 40 mg/kg/day and the LEL is 200 mg/kg/day.

The registrant must submit the purity of the test material before this evaluation can be considered complete.

B. CONCLUSION:

The NOEL for systemic toxicity in this study is 1000 mg/kg/day, the highest dose tested. The NOEL for dermal irritation is 40 mg/kg/day and the LEL is 200 mg/kg/day. The indicated dose levels are tentative, depending on the purity of the Banvel Herbicide, which was not provided in the report.

Core-classification: supplementary (can be upgraded if the purity of the test material is provided)

TABLE 3

PROJECT NO.:WIL-15143 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DANKEL HERBICIDE - PAGE 2

SPONSOR:VELSICOL CHEMICAL CORP. DERMAL OBSERVATIONS: TOTAL INCIDENCE / NO. ANIMALS

—— M A L E ——

TABLE RANGE:	DAY 1 TO DAY 13			
GROUP:	1	2	3	4
DERMAL OBSERVATIONS				
-SCORED, NOT REMARKABLE	35/ 5	27/ 5	25/ 5	6/ 4
-NO ERYTHEMA	0/ 0	0/ 0	0/ 0	1/ 1
-ERYTHEMA - VERY SLIGHT	0/ 0	0/ 2	10/ 4	14/ 5
-ERYTHEMA - SLIGHT	0/ 0	0/ 0	0/ 0	13/ 5
-ERYTHEMA - MODERATE	0/ 0	0/ 0	0/ 0	1/ 1
-NO EDEMA	0/ 0	0/ 2	0/ 4	13/ 4
-EDEMA - VERY SLIGHT	0/ 0	0/ 0	2/ 1	5/ 2
-EDEMA - SLIGHT	0/ 0	0/ 0	0/ 0	9/ 4
-EDEMA - MODERATE	0/ 0	0/ 0	0/ 0	2/ 1
-DESQUAMATION	0/ 0	1/ 1	1/ 1	9/ 3
-FISSURING	0/ 0	0/ 0	0/ 0	7/ 2

1- 0 MG/KG/DAY 2- 40 MG/KG/DAY 3- 200 MG/KG/DAY 4- 1000 MG/KG/DAY

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TABLE 3

PROJECT NO.:WIL-15163 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DARVEL HERBICIDE PAGE 3
SPONSOR:VELSICOL CHEMICAL CORP. DERMAL OBSERVATIONS: TOTAL INCIDENCE / NO. ANIMALS

—— M A L E ——

TABLE RANGE:	DAY 14 TO DAY 20 D			
GROUP:	1	2	3	4
DERMAL OBSERVATIONS				
-SCORED, NOT REMARKABLE	35/ 5	29/ 5	12/ 3	0/ 0
-NO ERYTHEMA	0/ 0	0/ 0	1/ 1	0/ 0
-ERYTHEMA - VERY SLIGHT	0/ 0	4/ 4	21/ 4	25/ 5
-ERYTHEMA - SLIGHT	0/ 0	0/ 0	1/ 1	9/ 3
-ERYTHEMA - MODERATE	0/ 0	0/ 0	0/ 0	1/ 1
-NO EDEMA	0/ 0	5/ 4	14/ 4	13/ 4
-EDEMA - VERY SLIGHT	0/ 0	1/ 1	7/ 3	13/ 5
-EDEMA - SLIGHT	0/ 0	0/ 0	0/ 0	7/ 3
-EDEMA - MODERATE	0/ 0	0/ 0	0/ 0	2/ 1
-DESQUAMATION	0/ 0	0/ 0	2/ 1	15/ 3
-FISSURING	0/ 0	0/ 0	1/ 1	2/ 2

1- 0 MG/KG/DAY 2- 40 MG/KG/DAY 3- 200 MG/KG/DAY 4- 1000 MG/KG/DAY
B- REPRESENTS THE LAST RECORDED DERMAL OBSERVATION FOR ALL ANIMALS IN THE STUDY.

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App. B, 2

TABLE 3

PROJECT NO.:WIL-15163 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH BANVEL HERBICIDE PAGE 6

SPONSOR:VELSICOL CHEMICAL CORP. DERMAL OBSERVATIONS: TOTAL INCIDENCE / NO. ANIMALS

----- F E M A L E -----

TABLE RANGE:	DAY 7 TO DAY 13			
GROUP:	1	2	3	4
DERMAL OBSERVATIONS				
-SCORED, NOT REMARKABLE	35/ 5	28/ 5	16/ 4	3/ 1
-ERYTHEMA - VERY SLIGHT	0/ 0	7/ 3	13/ 5	17/ 5
-ERYTHEMA - SLIGHT	0/ 0	0/ 0	6/ 3	13/ 4
-ERYTHEMA - MODERATE	0/ 0	0/ 0	0/ 0	2/ 1
-NO EDEMA	0/ 0	7/ 3	19/ 5	17/ 4
-EDEMA - VERY SLIGHT	0/ 0	0/ 0	0/ 0	7/ 4
-EDEMA - SLIGHT	0/ 0	0/ 0	0/ 0	6/ 3
-EDEMA - MODERATE	0/ 0	0/ 0	0/ 0	2/ 1
-DESQUAMATION	0/ 0	1/ 1	4/ 4	19/ 5
-FISSURING	0/ 0	0/ 0	0/ 0	13/ 3
-ATONIA	0/ 0	0/ 0	0/ 0	4/ 2

1- 0 MG/KG/DAY 2- 40 MG/KG/DAY 3- 200 MG/KG/DAY 4- 1000 MG/KG/DAY

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TABLE 3

PROJECT NO.:MIL-15163 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DARVEL MEMORIDE PAGE 7

SPONSOR:VELSICOL CHEMICAL CORP. DERMAL OBSERVATIONS: TOTAL INCIDENCE / NO. ANIMALS

---- F E M A L E ----

TABLE RANGE:	DAY 14 TO DAY 20 D			
GROUP:	1	2	3	4
DERMAL OBSERVATIONS				
-SCORED, NOT REMARKABLE	5/ 5	20/ 5	11/ 4	1/ 1
-ERYTHEMA - VERY SLIGHT	0/ 0	7/ 3	22/ 5	19/ 5
-ERYTHEMA - SLIGHT	0/ 0	0/ 0	2/ 1	15/ 4
-NO EDEMA	0/ 0	7/ 3	16/ 5	8/ 3
-EDEMA - VERY SLIGHT	0/ 0	0/ 0	0/ 2	19/ 5
-EDEMA - SLIGHT	0/ 0	0/ 0	0/ 0	0/ 3
-EDEMA - MODERATE	0/ 0	0/ 0	0/ 0	1/ 1
-DESQUAMATION	0/ 0	0/ 0	1/ 1	24/ 5
-FISSURING	0/ 0	0/ 0	1/ 1	4/ 4

1- 0 MG/KG/DAY 2- 40 MG/KG/DAY 3- 200 MG/KG/DAY 4- 1000 MG/KG/DAY

D- REPRESENTS THE LAST RECORDED DERMAL OBSERVATION FOR ALL ANIMALS IN THE STUDY.

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TABLE 5

PROJECT NO.:HIL-15143 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DANUEL HERBICIDE PAGE 1

SPONSOR:VELSICOL CHEMICAL CORP. WEEKLY BODY WEIGHT GAINS (GRAMS) - SUMMARY OF MEANS

		MALE			
	GROUP:	0 MG/KG/DAY	40 MG/KG/DAY	200 MG/KG/DAY	1000 MG/KG/DAY
WEEK	0 TO 1				
	MEAN	222.0	305.4	241.2	208.4
	S.D.	108.4	79.5	104.1	78.7
	N	5	5	5	5
	1 TO 2				
	MEAN	194.8	120.6	56.4	148.8
	S.D.	100.3	41.7	232.7	64.6
	N	5	5	5	5
	2 TO 3				
	MEAN	112.4	7.6	74.8	8.4
	S.D.	77.2	147.2	49.4	93.7
	N	5	5	5	5

NONE SIGNIFICANTLY DIFFERENT USING DUNNETT'S TEST

TABLE 5

PROJECT NO.:HRL-15143 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DAMMEL HERBICIDE PAGE 2

SPONSOR:VELSICOL CHEMICAL CORP. WEEKLY BODY WEIGHT GAINS (GRAMS) - SUMMARY OF MEANS

		FEMALE			
	GROUP:	0 MG/KG/DAY	40 MG/KG/DAY	200 MG/KG/DAY	1000 MG/KG/DAY
WEEK 0 TO 1					
	MEAN	268.8	247.8	240.6	264.4
	S.D.	108.0	86.9	96.7	86.7
	N	5	5	5	5
1 TO 2					
	MEAN	238.0	225.4	184.2	139.2@
	S.D.	48.4	30.4	77.3	67.8
	N	5	5	5	5
2 TO 3					
	MEAN	111.2	27.6	70.0	102.8
	S.D.	53.9	137.7	29.8	43.7
	N	5	5	5	5

@ = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP 1 AT 0.05 LEVEL USING DUNNETT'S TEST

TABLE 4

PROJECT NO.:MDL-15143 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DIAMEL HERBICIDE PAGE 1

SPONSOR:VELSICOL CHEMICAL CORP. WEEKLY BODY WEIGHTS (GRAMS) - SUMMARY OF MEANS

			MALE		
GROUP:	0 MG/KG/DAY	40 MG/KG/DAY	200 MG/KG/DAY	1000 MG/KG/DAY	
WEEK 0					
MEAN	2156.4	2144.4	2166.4	2174.8	
S.D.	104.8	121.9	92.3	136.3	
N	5	5	5	5	
1					
MEAN	2478.4	2449.8	2387.4	2413.2	
S.D.	84.8	170.5	117.4	150.8	
N	5	5	5	5	
2					
MEAN	2473.2	2570.4	2564.0	2564.0	
S.D.	113.1	151.7	211.3	201.9	
N	5	5	5	5	
3					
MEAN	2785.4	2570.0	2638.0	2564.4	
S.D.	63.2	206.0	232.2	240.4	
N	5	5	5	5	

NONE SIGNIFICANTLY DIFFERENT USING DUNNETT'S TEST

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TABLE 4

PROJECT NO.:HDL-15143 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DARVEL MEDICINE PAGE 2

SPONSOR:VELSICOL CHEMICAL CORP. WEEKLY BODY WEIGHTS (GRAMS) - SUMMARY OF MEANS

		— FEMALE —			
	GROUP:	0 MG/KG/DAY	40 MG/KG/DAY	200 MG/KG/DAY	1000 MG/KG/DAY
WEEK 0					
	MEAN	2114.8	2124.4	2117.2	2125.2
	S.D.	100.5	73.5	83.0	122.8
	N	5	5	5	5
1					
	MEAN	2383.6	2494.2	2457.8	2389.6
	S.D.	131.0	148.4	39.7	114.9
	N	5	5	5	5
2					
	MEAN	2641.6	2719.4	2642.0	2528.8
	S.D.	163.9	155.0	89.7	194.1
	N	5	5	5	5
3					
	MEAN	2752.8	2747.2	2712.0	2631.6
	S.D.	138.6	88.1	76.9	206.1
	N	5	5	5	5

NONE SIGNIFICANTLY DIFFERENT UNDER DUNNETT'S TEST

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TABLE 11

PROJECT NO.:WIL-15143 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DANKEL HERBICIDE PAGE 1
SPONSOR:VELSICOL CHEMICAL CORP. ORGAN WEIGHTS (G): SUMMARY OF MEANS WEEK 3

SACRIFICE NUMBER: 1

		MALE			
GROUP:		0 MG/KG/DAY	40 MG/KG/DAY	200 MG/KG/DAY	1000 MG/KG/DAY
TESTES					
	MEAN	2.8	3.5	3.4	2.7
	S.D.	0.27	0.85	0.40	0.83
	N	5	5	5	5
KIDNEY					
	MEAN	17.9	16.9	17.5	16.2
	S.D.	2.00	2.18	2.69	2.54
	N	5	5	5	5
LIVER					
	MEAN	104.4	95.1	87.2	81.8
	S.D.	14.29	17.31	12.38	13.36
	N	5	5	5	5

NONE SIGNIFICANTLY DIFFERENT USING DUNNETT'S TEST

TABLE 11

PROJECT NO.:WIL-15143 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DANGEL HERBICIDE PAGE 2
SPONSOR:VELSICOL CHEMICAL CORP. ORGAN WEIGHTS (G): SUMMARY OF MEANS WEEK 3

SACRIFICE NUMBER: 1

		---- FEMALE ----			
GROUP:		0 MG/KG/DAY	40 MG/KG/DAY	200 MG/KG/DAY	1000 MG/KG/DAY
OVARIES					
	MEAN	0.239	0.277	0.209	0.271
	S.D.	0.0900	0.1008	0.0336	0.1448
	N	4	5	5	5
KIDNEY					
	MEAN	16.2	16.0	15.1	16.4
	S.D.	2.01	1.78	1.34	1.83
	N	4	5	5	5
LIVER					
	MEAN	108.1	96.2	91.5	89.0
	S.D.	18.68	14.46	15.57	12.62
	N	4	5	5	5

NONE SIGNIFICANTLY DIFFERENT USING DUNNETT'S TEST

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TABLE 12

PROJECT NO.:HIL-15163 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DANGEL HERBICIDE PAGE 1
SPONSOR:VELSICOL CHEMICAL CORP. ORGAN WEIGHTS RELATIVE TO FINAL BODY WEIGHTS (G/100 G) WEEK 3

SACRIFICE NUMBER: 1

		MALE			
GROUP:		0 MG/KG/DAY	40 MG/KG/DAY	200 MG/KG/DAY	1000 MG/KG/DAY
TESTES					
	MEAN	0.097	0.133	0.126	0.104
	S.D.	0.0103	0.0245	0.0169	0.0341
	N	5	5	5	5
KIDNEY					
	MEAN	0.433	0.454	0.458	0.426
	S.D.	0.0432	0.0413	0.0760	0.0332
	N	5	5	5	5
LIVER					
	MEAN	3.752	3.653	3.252	3.168
	S.D.	0.3427	0.2424	0.3734	0.3871
	N	5	5	5	5

NONE SIGNIFICANTLY DIFFERENT USING BARNETT'S TEST

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TABLE 12

PROJECT NO.:WIL-13163 — 21-DAY DERMAL TOXICITY STUDY IN RABBITS WITH DANUEL HERBICIDE — PAGE 2
SPONSOR:HELSICOL CHEMICAL CORP. — ORGAN WEIGHTS RELATIVE TO FINAL BODY WEIGHTS (G/100 G) — WEEK 3

SACRIFICE NUMBER: 1

GROUP:	0 MG/KG/DAY	40 MG/KG/DAY	200 MG/KG/DAY	1000 MG/KG/DAY
		—— FEMALE ——		
OVARIES				
MEAN	0.008	0.010	0.008	0.010
S.D.	0.0030	0.0041	0.0020	0.0009
N	4	5	5	5
KIDNEY				
MEAN	0.579	0.565	0.556	0.619
S.D.	0.0476	0.0409	0.0447	0.0704
N	4	5	5	5
LIVER				
MEAN	3.833	3.410	3.359	3.340
S.D.	0.4700	0.5008	0.4858	0.3175
N	4	5	5	5

NONE SIGNIFICANTLY DIFFERENT USING DUNNETT'S TEST

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Combined Neoplastic & Non-Neoplastic Incidence For Died On Study and Scheduled Sacrifice Animals
Valsical Chemical Corporation
28-Day Dermal Toxicity Study in Rabbits with Banvel Herbicide

Project Number: WIL-15153 Species: Rabbit

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Tissue/ Diagnosis/ Modifier	Group 1		Group 2		Group 3		Group 4	
	M	F	M	F	M	F	M	F
Kidney	(5)	(5)	(0)	(0)	(0)	(0)	(5)	(5)
Within normal limits	3	1	0	0	0	0	3	3
Mineralization	1	4	0	0	0	0	1	2
minimal	0	3	0	0	0	0	1	2
mild	0	1	0	0	0	0	0	0
moderate	1	0	0	0	0	0	0	0
Nephritis, interstitial, chronic	0	0	0	0	0	0	1	1
mild	0	0	0	0	0	0	1	1
Vacuolation, conv. tub. epithelium cytoplasmic	1	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
mild	1	0	0	0	0	0	0	0
Liver	(5)	(5)	(0)	(0)	(0)	(0)	(5)	(5)
Within normal limits	5	5	0	0	0	0	4	2
Hepatocyte vacuolation, centrilobular	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Portal mononuclear cell infiltrate	0	0	0	0	0	0	1	2
minimal	0	0	0	0	0	0	1	1
moderate	0	0	0	0	0	0	0	1
Skin (treated)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Within normal limits	5	3	3	4	2	2	3	0
Acanthosis	0	2	2	1	2	2	1	3
minimal	0	2	2	1	2	1	1	1
mild	0	0	0	0	0	1	0	1
moderate	0	0	0	0	0	0	0	1
Dermatitis, chronic	0	1	0	0	0	0	0	0
mild	0	1	0	0	0	0	0	0
Hyperkeratosis	0	0	1	0	2	3	2	5
minimal	0	0	1	0	2	1	0	0
mild	0	0	0	0	0	2	1	3
moderate	0	0	0	0	0	0	1	2
Inflammation, chronic active	0	0	0	1	0	0	0	0
minimal	0	0	0	1	0	0	0	0
Ulcer	0	0	0	0	1	0	0	0
minimal	0	0	0	0	1	0	0	0
Skin (untreated)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Within normal limits	5	5	5	5	5	5	5	5

Titles:
Group 1 Control (deionized water)
Group 2 10 mg/kg/day Banvel Herbicide
Group 3 200 mg/kg/day Banvel Herbicide
Group 4 1000 mg/kg/day Banvel Herbicide

() = Total Examined

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Microscopic Incidence Page: 1

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END